

In re Application of:
Fernandez et al.
Application No.: 09/990,091
Filed: November 21, 2001
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PATENT
Atty. Docket No.: INVIT1120-3

EXHIBIT B

Pending Claims Upon Entry of the Amendment

39. (Previously added) A method for producing a library of expressible open reading frames, the method comprising:

- a) amplifying deoxyribonucleic acid (DNA) molecules comprising a plurality of open reading frames (ORFs) using a primer pair, wherein the primer pair comprises a 5' primer, which comprises a nucleotide sequences starting 5'-CACCATG, thereby producing a plurality of amplified ORFs;
- b) inserting amplified ORFs of the plurality into an expression vector, thereby producing expression vectors comprising the amplified ORFs; and
- c) verifying the size and orientation of the amplified ORFs in the expression vectors, thereby producing a library of expressible ORFs.

40. (Previously added) The method of claim 39, wherein the primer pair further comprises a 3' primer, which causes the amplification product to end at the third position of a codon immediately preceding a stop codon of an ORF being amplified.

41 (Previously added). The method of claim 40, wherein the 3' primer further causes the amplification product to comprise a 3' terminal adenine residue.

42. (Previously added) The method of claim 39, further comprising transforming cells with the expression vectors comprising the amplified ORFs, thereby obtaining a library of transformed cells containing the expression vectors.

43. (Previously added) The method of claim 39, further comprising purifying the amplified ORFs prior to inserting the amplified ORFs into the expression vector.

44. (Previously added) The method of claim 43, wherein purifying the amplified ORFs is performed using column chromatography or gel electrophoresis.



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45. (Previously added) The method of claim 43, wherein purifying the amplified ORFs is performed using agarose gel electrophoresis.

46. (Previously added) The method of claim 45, wherein the agarose is low melt agarose.

47. (Previously added) The method of claim 39, wherein amplified ORFs of the plurality encode full length proteins.

48. (Previously added) The method of claim 39, wherein inserting the amplified ORFs into the expression vector is performed using an enzyme that cleaves and ligates DNA.

49. (Previously added) The method of claim 48, wherein the enzyme is a type I topoisomerase or a site-specific recombinase.

50. (Previously added) The method of claim 48, wherein the enzyme is a vaccinia DNA topoisomerase, a lambda integrase, an FLP recombinase, or a P1-Cre protein.

51. (Previously added) The method of claim 48, wherein the enzyme is a vaccinia DNA topoisomerase.

52. Cancelled

53. Cancelled

54. (Previously added) The method of claim 39, wherein the expression vector is suitable for prokaryotic expression and eukaryotic expression.

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55. (Previously added) The method of claim 39, wherein verifying the size and orientation of the ORF is performed using a polymerase chain reaction protocol.

56. (Previously added) The method of claim 40, wherein verifying the size and orientation of the ORF is performed using whole cell lysates of transformed cells containing the expression vectors.

57. (Previously added) The method of claim 39, wherein the DNA molecules comprise prokaryotic DNA or eukaryotic DNA.

58. Cancelled

59. (Previously added) The method of claim 39, wherein the amplified ORFs of the plurality encode members of a family of proteins.

60. (Previously added) The method of claim 59, wherein the members of the family of proteins are human proteins.

61. (Previously added) The method of claim 59, wherein the members of the family of proteins comprises members of a family of kinases, phosphatases, transcription factors, oncogenes, or tumor suppressors.

62. (Previously added) The method of claim 39, which is performed in a high throughput format.

63. (Previously added) The method of claim 39, which is performed in a multiwell microtiter plate.

64. (Previously added) The method of claim 40, further comprising the additional step of verifying the size and orientation of the ORF in the expression vector in the transformed cells.

65. (Previously added) The method of claim 40, wherein the transformed cells are eukaryotic cells or prokaryotic cells.

66. (Previously added) The method of claim 40, wherein the transformed cells are bacteria, yeast, fungi, insect cells, mammalian cells, or plant cells.

67. Cancelled

68. (Previously added) The method of claim 39, wherein the expression vector comprises a nucleotide sequence encoding an affinity purification tag or an epitope tag, and wherein the expressible ORF and the nucleotide sequence encode a fusion protein comprising a polypeptide encoded by the ORF and the tag.

69. (Previously added) A library of expressible ORFs produced according to the method of claim 39.

70. Cancelled

71. (Previously added) A method for producing a library of selected expressible open reading frames (ORFs), the method comprising:

- 66, 67, 68, 69, 70, 71*
- a) amplifying deoxyribonucleic acid (DNA) molecules comprising a plurality of ORFs using a primer pair, wherein the primer pair comprises a 5' primer, which comprises a nucleotide sequences starting 5'-CACCATG, and a 3' primer, which causes the amplification product to end just prior to a stop codon, thereby producing a plurality of amplified ORFs;
 - b) purifying amplified ORFs of the plurality, thereby obtaining purified amplified ORFs;
 - c) inserting the purified amplified ORFs into expression vectors using a vaccinia DNA topoisomerase, thereby producing expression vectors comprising the amplified ORFs;
 - d) transforming cells with the expression vectors comprising the amplified ORFs; and

e) selecting transformed cells containing expression vectors comprising ORFs in an orientation for expression of a polypeptide encoded by the ORF.

72. (Previously added) The method of claim 71, wherein purifying the amplified ORFs comprises separating the amplified ORFs using agarose gel electrophoresis, and isolating the amplified ORFs from the agarose gel.

73. (Previously added) The method of claim 72, wherein the agarose is low melt agarose.

74. (Currently amended) The method of claim 71, wherein inserting the purified amplified ORFs into the expression vectors is performed using an enzyme that cleaves and ligates DNA.

75. (Previously added) The method of claim 74, wherein the enzyme is a vaccinia DNA topoisomerase, a lambda integrase, an FLP recombinase, or a P1-Cre protein.

76. (Previously added) The method of claim 74, wherein the expression vectors are suitable for prokaryotic expression and eukaryotic expression.

77. (New) A method for producing a library of selected expressible open reading frames (ORFs), the method comprising:

a) amplifying deoxyribonucleic acid (DNA) molecules comprising a plurality of ORFs using a primer pair, wherein the primer pair comprises a 5' primer, which comprises a nucleotide sequences starting 5'-CACCATG, and a 3' primer, which causes the amplification product to end just prior to a stop codon, thereby producing a plurality of amplified ORFs;

b) inserting amplified ORFs of the plurality into expression vectors using a vaccinia DNA topoisomerase, thereby producing expression vectors comprising the amplified ORFs;

c) transforming cells with the expression vectors comprising the amplified ORFs;
and

d) selecting transformed cells containing expression vectors comprising ORFs in
an orientation for expression of a polypeptide encoded by the ORF.

78. (New) The method of claim 77, wherein inserting the amplified ORFs into the
expression vectors is performed using an enzyme that cleaves and ligates DNA.

79. (New) The method of claim 78, wherein the enzyme is a vaccinia DNA
topoisomerase, a lambda integrase, an FLP recombinase, or a P1-Cre protein.

80. (New) The method of claim 77, wherein the expression vectors are suitable for
prokaryotic expression and eukaryotic expression.